

TRANSPORT OF ^{14}C -INDOLEACETIC ACID ACROSS PEDICEL ABSCISSION ZONES IN *HIBISCUS*

LINDA J. NASH, CHRIS H. BORNMAN AND FREDRICK T. ADDICOTT*

(Department of Botany, University of Natal, Pietermaritzburg, South Africa)

ABSTRACT

Studies using ^{14}C -indoleacetic acid (IAA) as a tracer in *Hibiscus* pedicel explants indicate that non-physiological concentrations of the hormone do not diffuse freely down the pedicel but tend to be immobilised by the tissue, especially at the pedicellar abscission zone. Transport at the terminal stage includes an acropetal component. Indication is that the tissue primarily responsible for movement of this hormone may be the cortical parenchyma. Transport varies directly with tissue age and inversely with IAA concentration.

UITTREKSEL

VERVOER VAN ^{14}C -INDOOLASYNSUUR OOR DIE AFSNYDINGSONE IN DIE
BLOMSTEELE VAN *HIBISCUS*.

Studies met radioaktiewe indoolasynsuur (IAS) op blomsteeleksplante van *Hibiscus* toon dat nie-fisiologiese konsentrasies van dié hormoon nie vryelik in die blomsteel af diffundeer nie maar dat dit veral by die afsnydingstreek geïmmobiliseer word. Aanvanklik blyk dit dat vervoer van die hormoon 'n akropetale komponent insluit. Daar is 'n aanduiding dat die kortikale parenchium dié weefsel is waarin die hormoon hoofsaaklik beweeg. Vervoer varieer direk met ouderdom van die weefsel en omgekeerd met konsentrasie van die IAS.

INTRODUCTION

Abscission of fertilised and unfertilised ovaries in *Hibiscus* takes place at a well-defined abscission zone approximately 1 cm below the ovary. The zone is morphologically distinct as a constriction in the pedicel (Fig. 1) and is apparently present at the primordial stage of floral development. Since both floral buds and fruits are sources of endogenous auxin (Fawcett, 1961) which at certain concentrations acts as an abscission retardant (Addicott and Lynch, 1955), a transport study was undertaken in order to determine whether the abscission zone in *Hibiscus* presents a physical barrier to its free basipetal transport.

In a second series of experiments, the effects of age of tissue and of concentration of applied auxin were investigated, with the following in mind: firstly, the anatomy of the abscission process suggests that the transport properties of the tissue probably are age-dependent; and secondly, if transport is dependent on a limited number of transport sites as suggested by Leopold and de la Fuente (1968), the behaviour of high concentrations of hormone should differ from that of lower concentrations.

* Current address: Department of Agronomy and Range Science, University of California, Davis, Calif.

Accepted for publication 18th June, 1971.

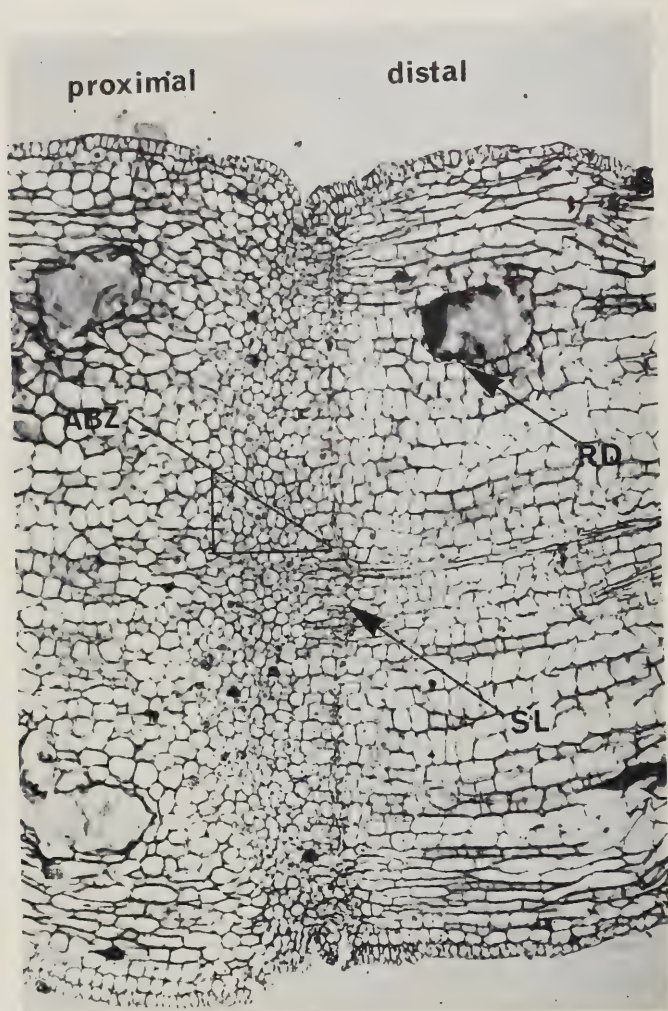


FIG. 1.

Longitudinal section of pedicel abscission zone at stage when corolla is fully extended, corresponding to Fig. 2b.

ABZ, abscission zone; RD, resin duct; SL, separation layer.

Since Jacobs and McCready (1967) showed that a cylinder of pith parenchyma of *Coleus* transported auxin at essentially the same velocity as a cylinder including vascular tissue, it was hoped also to gain some understanding of auxin movement in relation to type of tissue in the *Hibiscus* pedicel.

MATERIALS AND METHODS

A carboxyl-labelled indoleacetic acid solution was monitored by liquid-scintillation counting. Transport was studied by means of the conventional agar donor-receiver block system (Kaldewey, 1968). Pedicels were selected from a single specimen shrub of *Hibiscus rosa-sinensis*, a hybrid in which seed is not set, as far as possible from the same area of the plant and at the same time of day. Pedicels of three different ages were selected, their age being determined by the appearance of the subtended flower (Fig. 2). Pedicels of median age (Fig. 2b), that is with fully extended corollas, were used for the initial transport experiments while pedicels from tightly-closed buds (Fig. 2a), and from ovaries which had shed their corollas (Fig. 2c), respectively, were used for later experiments in which the effects of age were investigated.

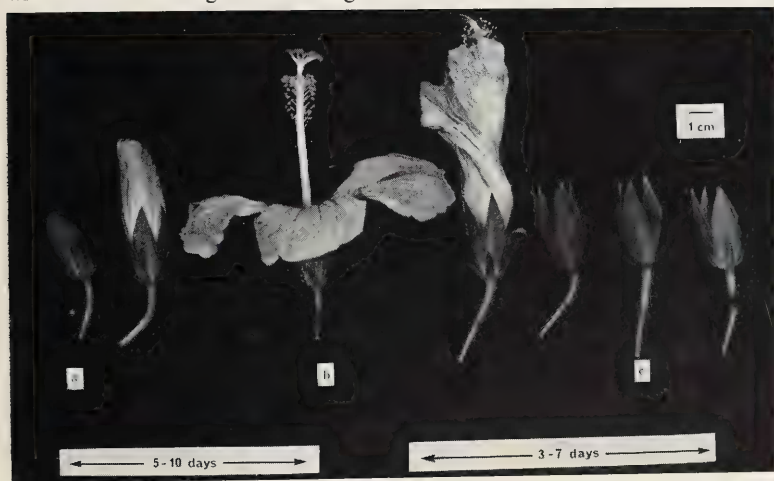


FIG. 2.

Floral development in *Hibiscus rosa-sinensis*.
a, bud stage; b, mature stage; and c, pre-abscission stage.

Identical 9 mm-pedicel segments were cut and immediately placed with the morphological apex up between similar 50 mm³ blocks of 3 per cent agar, to the upper of which was then applied a 20- μl droplet of ^{14}C -indoleacetic acid (^{14}C -IAA) solution. This block, the donor, was replaced in later experiments by a

block in which an identical amount of labelled auxin had been incorporated during preparation of the agar; this technique was found to give improved reproducibility. In early experiments, where the explants were placed with the morphological base upward, no downward transport of radio-activity was detectable above the background value after 60 minutes.

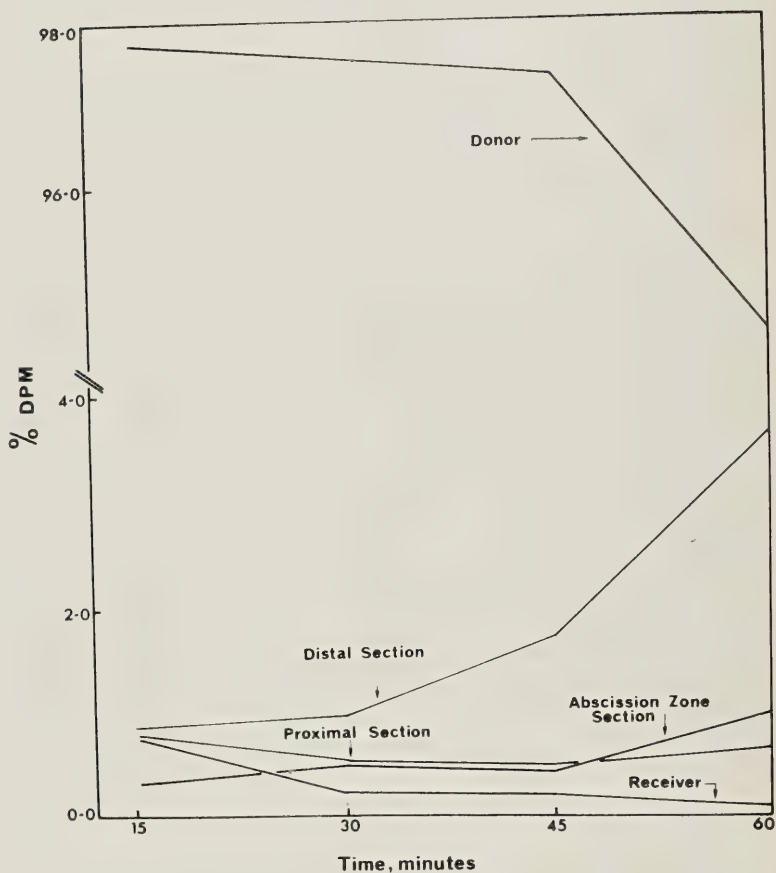


FIG. 3.

Distribution of radioactivity in donor-receiver system during a 60-minute transport period.

The ^{14}C -IAA solution used for the initial experiments had an activity of ca. 1.0×10^4 dpm (5.0 nCi) per $10 \mu\text{l}$; this solution was used in subsequent experiments as the "high-activity" solution as opposed to a "low-activity" solution of ca. 4.5×10^2 dpm (0.2 nCi) per $10 \mu\text{l}$.

After the duration of a specified transport period, the donor and receiver blocks were removed and each dissolved in 15 ml scintillation medium in a vial. The explant was then cut into three identical 3 mm segments, the median of which thus included the abscission zone, and each was macerated in 2 ml absolute ethanol in a vial for a standard period of 5 minutes. In studies involving extractable and non-extractable auxin, the tissue debris and the supernatant ethanol were separated at this stage. Fifteen ml scintillation medium was then added to each vial. The scintillation medium consisted of: naphthalene AR 60 g, PPO 4 g, dimethyl POPOP 200 mg, absolute ethanol 125 ml, ethylene glycol 20 ml, and toluene AR to 1 000 ml.

Activity per sample was counted in a Nuclear-Chicago Unilux II liquid scintillation counter and, after correcting for counting efficiency, expressed as a percentage of the system's total activity. The petri dishes which had been in contact with the receiver blocks were washed with ethanol and the washings examined for activity; in no case, however, did this exceed the atmospheric background count by more than 10%. These values were added to those obtained from the receiver blocks.

Sections of tissue, 5–10 μ thick, containing 1–2 nCi per ml of ^{14}C were exposed to an autoradiographic emulsion for 4–5 days in an attempt to localise the sites of radioactive auxin.

RESULTS AND DISCUSSION

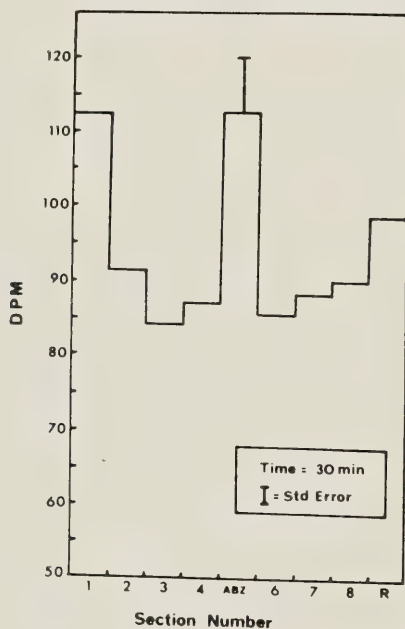
Figures 3, 4 and 5 represent the results obtained in the preliminary transport experiments whereas Figs. 6 and 7 show the effects of age and hormone concentration on the transport system. Additional data from these experiments are summarised, in the accompanying table.

Results such as those shown in Fig. 4 indicate a small accumulation of auxin at the abscission zone, although comparison with Fig. 3 may suggest that this accumulation might be due to the basipetal transport of a concentration peak. Nevertheless, simple polar transport of a downward-moving pulse does not provide an adequate explanation for these results. Firstly, the decrease in activity of the donor in Fig. 3 is not matched by an increase in the receiver in; fact the activity of the receiver decreases after 15 minutes transport. Furthermore, Fig. 5 shows that an appreciable amount of the applied activity is not ethanol-extractable and remains associated with the tissue debris; it is thus effectively removed from the transport system.

TABLE 1.
Comparison between transport capacities of tissues of different ages.

Tissue/ Treatment	T(min)	Section (Activity as % dpm)				
		Donor	Distal	Abscission Zone	Proximal	Receiver
o/l	15	22,9	15,6	13,4	29,5	18,4
	30	30,2	17,3	19,7	16,6	18,0
y/l	15	89,5	0,4	8,6	0,5	0,9
	30	66,2	9,3	10,8	10,1	13,4
o/h	15	63,5	41,3	15,0	1,4	5,8
y/h	15	89,0	7,5	1,4	0,7	1,3
m/h	5	99,1	0,2	0,2	0,2	0,2
	15	97,6	0,7	0,5	0,4	0,7
	30	97,4	0,9	0,5	0,4	0,6
	45	97,1	1,8	0,4	0,3	0,3
	60	95,8	3,4	0,3	0,2	0,2

o, old tissue; m, mature tissue; y, young tissue; h, high applied auxin; l, low applied auxin



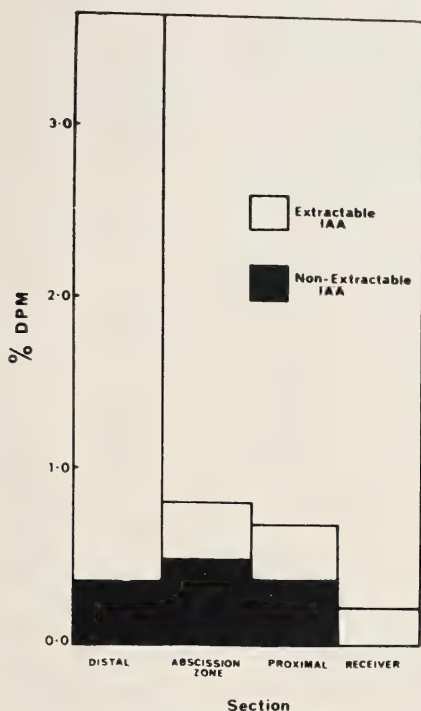


FIG. 5.

Radioactivity of extractable and non-extractable auxin after a 60-minute transport period.

The distributions shown in Figs. 6 and 7 suggest the following relationship between tissue age, auxin concentration, and rate of transport:

- (i) very young pedicels are resistant to IAA transport, as is evident from the slow decrease in activity of the donor blocks;
- (ii) in explants from older pedicels, however, there is a relatively rapid distribution of activity, suggesting that the tissue offers little resistance to free basipetal transport of IAA;
- (iii) low auxin concentrations are distributed more rapidly than high ones in tissue of comparable age; however, high concentrations in old tissue are distributed more rapidly than low concentrations in young tissue;

FIG. 4.

Localisation of radioactivity in 1-mm sections after a transport period of 30 minutes. ABZ, abscission zone.

- (iv) there is evidence of acropetal recycling (Goldsmith, 1969) and of enzymatic decarboxylation (Iversen and Aasheim, 1970) at the terminal step in both old and young tissue; and
- (v) comparison with data obtained from mature tissue (see table) indicates that this is still more resistant to basipetal transport of high auxin concentrations than is very young tissue; in addition, acropetal recycling also occurs.

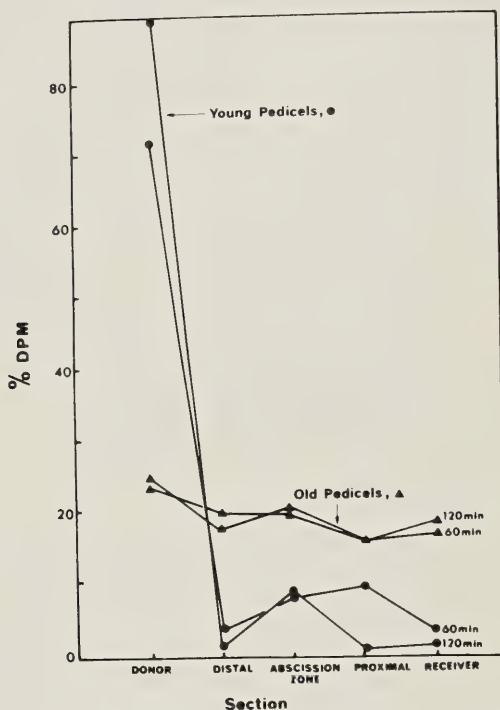


FIG. 6.

Transport profiles for low ^{14}C -IAA concentrations, that is 0,2 nCi/10 μl .

These observations are fully consistent with the view that abscission may be preceded by a reversal in auxin gradient across the abscission zone, and that the process includes a loss of cellular integrity.

A possible explanation for these results may be the following. During maturation and flowering, auxin is maintained at a high, abscission-retarding

level in the region distal to the abscission zone by the inactivation of the mechanism for basipetal transport between cells. Once abscission is initiated, however, this barrier is no longer operative and the auxin is freely transported across the abscission zone and towards auxin sinks in other organs.

A microscopic study of ^{14}C tracks in the developed autoradiographic emulsion in transverse sections of the pedicel, indicated a preference for the parenchyma of the cortex. However, a more definitive study is required to determine to what degree these tracks represent bound or free auxin, or degradation products.

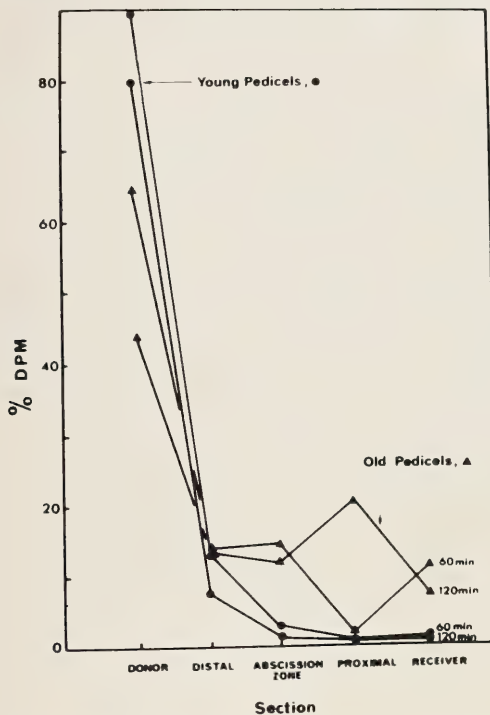


FIG. 7.

Transport profiles for high ^{14}C -IAA concentrations, that is $5.0 \text{ nCi}/10 \mu\text{l}$.

CONCLUSION

This study shows a small accumulatory effect in the pedicel abscission zone and some immobilisation, by metabolism or degradation, during auxin transport

down the pedicel. Some acropetal recycling and decarboxylation of auxin from the receiver block is also occurring. Transport is both age- and concentration-dependent. The highest transport values are obtained in old tissue to which low concentrations of ^{14}C -IAA are applied; the lowest, in mature tissue to which high concentrations of ^{14}C -IAA are applied. Very young tissue is more resistant to transport than old tissue, but less so than mature tissue.

ACKNOWLEDGMENTS

It gives us pleasure to acknowledge the Atomic Energy Board for supporting this research through capital and running expenses grants.

REFERENCES

- Addicott, F. T. and Lynch, R. S., 1955. Physiology of abscission. *Ann. Rev. Pl. Physiol.* **12**: 211–238.
- Fawcett, C. H., 1961. Indole auxins. *Ann. Rev. Pl. Physiol.* **12**: 345–368.
- Goldsmith, Mary Helen M., 1969. Transport of plant growth regulators. In: Wilkins, M.B., (ed.), *Physiology of Plant Growth and Development*. London: McGraw-Hill.
- Iversen, T. H. and Aasheim, T., 1970. Decarboxylation and transport of auxin in segments of sunflower and cabbage roots. *Planta* **93**: 354–362.
- Jacobs, W. P. and McCready, C. C., 1967. Polar transport of growth regulators in pith and vascular tissues of *Coleus* stems. *Amer. J. Bot.* **54**: 1035–1040.
- Kaldewey, H., 1968. Auxin transport: general remarks concerning terminology and the methods. In: Vardar, Y., (ed.), *The Transport of Plant Hormones*. Amsterdam: North Holland Publishing Co.
- Leopold, A. C. and de la Fuente, R. K., 1968. A view of polar auxin transport. In: Vardar, Y., (ed.), *The Transport of Plant Hormones*. Amsterdam: North Holland Publishing Co.